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Soil organic matter prevails over heavy metal pollution and vegetation as a factor shaping soil microbial communities at historical Zn–Pb mining sites

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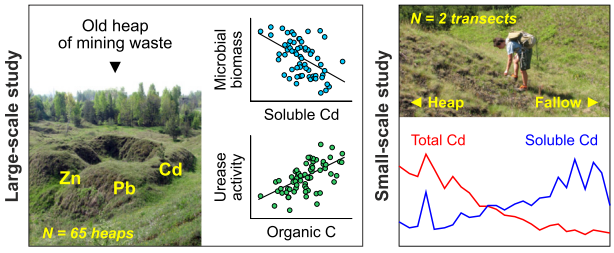
HIGHLIGHTS

- Mining waste heaps and surrounding fallows were highly polluted with Cd, Pb and Zn.
- Total or available heavy metals affected negatively soil microbial communities.
- Organic matter and nutrient availability had strongest positive effects on microbes.
- Plant species richness correlated positively with soil enzymes and mycorrhizal fungi.

GRAPHICAL ABSTRACT



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ABSTRACT

This study examined the effects of soil heavy metals, macronutrients, texture and pH as well as plant species richness and composition on soil respiration, enzymatic activity, microbial biomass, metabolic quotient (qCO_2) and arbuscular mycorrhizal fungi (AMF) at sites of historical Zn–Pb mining. The study was conducted both on a large scale (65 heaps scattered over the area of 750 km^2) and on a small scale (25 plots along two 48 m transects extending from heaps to adjacent fallow fields). Total concentrations of metals exceeded 400 (Cd), 20,000 (Pb) and 80,000 (Zn) mg kg^{-1} at the most polluted sites. Although they decreased along the heap-fallow direction, they still remained above environmental standards in fallow soils. In contrast, some soluble metal forms increased with the increasing distance from heaps. Soil organic matter had the strongest positive effect on most microbial parameters. Total and/or available heavy metals exhibited significant negative effects on microbial biomass, enzymatic activity and AMF, and a positive effect on qCO_2. Organic matter alleviated negative effects of heavy metals on microorganisms; they were not observed where the increase in the contamination was accompanied by the increase in organic matter content. Plant species richness affected positively enzymatic activity and mycorrhization level. Plant species composition possibly contributed to the formation of soil microbial communities, but its effect was entangled in that of heavy metals as plant communities changed along

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pollution gradients (from metal-tolerant grasslands dominated by *Festuca ovina* to calcareous grasslands and ruderal communities at less polluted sites).

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1. Introduction

Soil pollution with heavy metals is one of the most pressing environmental problems worldwide. It is caused largely by human activities, such as mining and processing of metal ores, combustion of fossil fuels, the use of fertilizers, including sewage sludge, and pesticides, road transport, and many industrial processes (Kabata-Pendias, 2011). Once released into the environment, heavy metals persist there for centuries or even millennia, disperse to distant areas and accumulate in biotic and abiotic components of ecosystems (Babst-Kostecka et al., 2018; Peralta-Videa et al., 2009; Pyatt et al., 2005). As a result, they can adversely affect human health and ecosystems for a long time after release and far from the source (Azarbad et al., 2013; El Khalil et al., 2008; Grattan et al., 2003; Moroń et al., 2012; Pyatt and Grattan, 2001).

The presence of heavy metals in the soil in amounts toxic to organisms is typically detected using bioindicators (Przybyłowicz et al., 2005; Żmudzki and Laskowski, 2012; Azarbad et al., 2013; Grześ et al., 2015; Woch et al., 2016). One of the most promising are microorganisms (Guo et al., 2017; Orłowska et al., 2002; Stefanowicz et al., 2009, 2008; Turnau et al., 2006). Firstly, they are in close contact with the environment due to a high surface-to-volume ratio, and thus are capable of responding quickly to changes in environmental conditions (Nielsen and Winding, 2002; Zhang et al., 2010). Secondly, they play a primary role in essential ecosystem processes such as organic matter decay, element cycling, soil structure formation and plant nutrition (Coleman et al., 2004). This means that any qualitative or quantitative changes in soil microbial communities caused by heavy metal pollution may translate into disturbances in the ecosystem functioning (Castro, 1997; Orłowska et al., 2002; Stefanowicz et al., 2009, 2008; Turnau et al., 2006).

Previous studies on field-contaminated and spiked soils have shown that elevated concentrations of heavy metals can affect the performance of microorganisms in different ways: reduce enzymatic activity, microbial biomass and functional diversity, i.e., the diversity of metabolic processes carried out by a microbial community, alter the taxonomic structure or increase the overall community tolerance (Stefanowicz et al., 2008, 2009; Gao et al., 2010; Azarbad et al., 2013; Chen et al., 2013; Guo et al., 2017). The sensitivity of soil microorganisms to heavy metals differs between microbial groups. Fungi are generally more resistant than bacteria and their activity and functional diversity may not change or even increase under the influence of metal contamination (Rajapaksha et al., 2004; Stefanowicz et al., 2008). Rhizobia are relatively sensitive to heavy metals, in contrast to nitrifiers and microbes involved in degradation of C compounds (Giller et al., 2009).

The variety of microbial responses may be a problem in detecting the effects of metal toxicity. This is especially true in field studies where these responses are modified by the presence of confounding factors. Some soil physicochemical properties, e.g., pH, texture, organic matter content, seem to be of primary importance (Azarbad et al., 2013; Chodak et al., 2013; Guo et al., 2017; Stefanowicz et al., 2012). They strongly affect microbial communities not only directly but also indirectly, by controlling the behaviour of heavy metals (Ciarkowska et al., 2014; Kabata-Pendias, 2011; Lauber et al., 2008). Metal mobility and bioavailability usually increase with decreasing pH and the content of organic matter and clay minerals (Kabata-Pendias, 2011; Lair et al., 2007; Stefanowicz et al., 2014). It should be realized, however, that these relationships are not always apparent due to complex connections between soil properties; for example, the increase in organic matter content may be accompanied by a decrease in pH (Stefanowicz et al., 2014) as a result of the increased concentration of organic (e.g., humic) acids.

Another factor that has a direct impact on soil microbial communities and is able to modify their response to heavy metals is vegetation (Eisenhauer et al., 2011; Gremion et al., 2004; Stefanowicz et al., 2012). Plants provide soil microorganisms with nutrients through litterfall and root exudates, the quantity and quality of which depend on the plant species composition. For example, plant litter rich in lignin and other phenolic compounds and characterized by a high C/N ratio decomposes slowly and supports fungal-dominated communities rather than those dominated by bacteria (Bugg et al., 2011; Mincheva et al., 2014; Paterson et al., 2008; Suseela et al., 2016). Studies conducted in post-industrial areas, in which bare and vegetated soils were compared, showed a positive effect of plant growth on microbial activity and the supply of soil nutrients (Stefanowicz et al., 2015; Borymski et al., 2018). Under pollution conditions, also plant diversity may be beneficial for soil microorganisms and processes they control (He et al., 2008; Maestre et al., 2012; Stefanowicz et al., 2012); the underlying mechanism is probably the same as described in non-pollution studies (Eisenhauer et al., 2011; Loranger-Merciris et al., 2006). Some authors concluded that plant cover and composition might be more important for soil microbial communities than heavy metal pollution (Stefanowicz et al., 2012; Pacwa-Płociniczak et al., 2018). Despite this, field studies on the impact of heavy metals on soil microorganisms typically neglect vegetation-related variables (Azarbad et al., 2013; Chodak et al., 2013; Kelly et al., 2003; Liao and Xie, 2007; Wang et al., 2007).

In this paper we evaluated the performance of soil microbial communities under severe and long-lasting heavy metal contamination caused by historical Zn–Pb mining activity in the Małopolska province, S Poland. Although old, abandoned land and facilities after mining operation (shafts, open pits, mining waste disposal sites) are a well-known environmental problem, they are rarely the subject of research. This is partly due to their unobtrusive nature. Over decades or even centuries, they naturally eroded, were overgrown by vegetation or mechanically destroyed (for example, levelled, where the area was intended for development), so that their remains are difficult to find nowadays. However, even if these places do not differentiate visually from the surrounding landscape, they can still pose a threat to human health and the environment due to the durability of metal pollution. Post-mining sites investigated in this work are old heaps of gangue (mining waste material) composed mainly of metal-rich dolomite and calcite. The heaps are small (they are usually several meters in diameter) and scattered over a large area. Nevertheless, their environmental impact should not be underestimated. First, the contents of heavy metals (Cd, Pb, Tl and Zn) in the soils developed on these heaps are high or extremely high compared to those reported for unpolluted soils (Stefanowicz et al., 2014). Even though these metals are largely immobilized due to the abundance of carbonate minerals, they can

be efficiently taken up by plants growing on heaps (Stefanowicz et al., 2016), and thus enter the trophic chains. Second, the examined heaps are situated in a densely populated area; many of them are near residential buildings and vegetable gardens as well as within arable fields. Therefore, they can locally pose a serious environmental risk. The soils of the above-described mining relics have never been the subject of systematic microbiological research. Hence, our goal was to assess basic microbial community parameters, such as basal respiration, microbial biomass, metabolic quotient, enzymatic activity and mycorrhization level. In addition, we aimed to determine how the level of metal stress influences microorganisms and to what extent this influence is modified by soil conditions (pH, texture, macroelement concentrations) and vegetation (species diversity and composition).

2. Materials and methods

2.1. Study area and fieldwork

The study area (ca. 750 km^2) was located in the south-eastern part of the Silesia-Cracow Upland (S Poland) between the towns of Jaworzno, Bolesław, Olkusz and Trzebinia (Stefanowicz et al., 2014). This region is known for its rich Zn–Pb ore deposits of Mississippi Valley type occurring in Triassic and Devonian rocks. The ores contain on average 4–6% Zn, 1–3% Pb and 5–8% Fe, as well as certain amounts of Cd, Tl, Ag and As (Cabala et al., 2009). The gangue minerals are largely composed of dolomite and calcite. Information on mining history, geology and mineralogy of the study area can be found in Cabala et al. (2009), Godzik and Woch (2015) and Woch (2015).

When investigating the effect of heavy metal pollution, we applied two approaches: 1) we used a gradient of soil concentrations of heavy metals formed by heaps (large-scale approach), and 2) we created a gradient of contamination by taking soil samples in transects covering the heap area and adjoining post-agricultural area, i.e., fallow field (small-scale approach). With a considerable variation in metal pollution, the first approach allowed maintaining approximately constant soil conditions (due to the mineralogical similarity of heap material), while the second approach allowed maintaining those conditions that are site-specific (e.g., microclimate, the pool of plant species).

The large scale approach encompassed 65 old heaps of mining waste located in open areas, mainly in agricultural and suburban landscape (a map of study sites was shown elsewhere; see Stefanowicz et al., 2014). A 4 m^2 plot was established on each heap in a homogenous patch of grassland vegetation. In each plot, the cover of vascular plant species was estimated on a 6-point scale (simplified Braun-Blanquet scale) in the 2012 and 2013 growing seasons. It was based on two visits per year to ensure that all species were recorded. In addition, the total percentage cover of vegetation was estimated with an accuracy of 5%. In May 2012, soil samples were taken from 3 spots located at the edge of each plot to a depth of 15 cm (after careful removal of the organic layer) and bulked into one composite sample per plot. As an overwhelming majority of plots were densely vegetated and thus the soil was intensively penetrated by plant roots, it may be assumed that soil samples also contained rhizosphere soil. The soil samples were kept in open plastic bags, transported immediately to the laboratory and deposited in a refrigerator at 4 °C.

For the small-scale approach, two heaps were selected. The first heap (near the town of Trzebinia) was located within an arable field, which had not been cultivated over the past several years, and showed signs of destruction due to agricultural works (there was no clear boundary between the heap and the surrounding field). The second heap, near the town of Bolesław, was in the agricultural area abandoned a few decades ago. In contrast to the first, it looked undisturbed (the boundary between the heap and the surrounding field was distinct). For each heap, a 48-m long transect was laid to cover as much as possible the entire heap and a part of the adjacent post-agricultural area (fallow field). Along each transect, 25 plots of 1 m^2 were established every 2 m. In the case of the Trzebinia transect, only 3 plots were within the heap (i.e., in its undisturbed part), while there were 9 such plots on the Bolesław transect. The vegetation surveys and the soil sample collection were carried out in transects as described above, for the large-scale approach.

2.2. Laboratory work

The level of heavy metal contamination was characterized by soil concentrations of Cd, Pb and Zn, because these elements have been most often indicated as exceeding the environmental standards in the study area (Babst-Kostecka et al., 2016; Krzaklewski et al., 2004; Pietrzykowski et al., 2011). All soil samples were analysed for the following physicochemical and microbial properties: pH, texture, organic C, total Ca, Cd, K, N, P, Pb and Zn, Olsen P, $BaCl_2$-extractable Ca, Cd, K, Pb and Zn, H_2O-extractable Cd, Pb and Zn, respiration, microbial biomass and urease and arylsulfatase activity. Additionally, in the first (large-scale) part of the study, the degree of arbuscular mycorrhizal fungi (AMF) colonization was assessed in plant roots found in the collected soil. The soil from each plot was homogenized by sieving (2 mm mesh) and split into two parts that were used either for physicochemical (kept at room temperature) or microbiological (kept at 4 °C) analyses.

2.2.1. Physicochemical analyses

Soils for physicochemical analyses were dried at 105 °C or at room temperature. Their texture was determined through a combination of sieving and sedimentation (ISO 11277, 1998), and pH with a pH-meter (Hach HQ40d) after extraction with water at a 1:5 (w:v) ratio (ISO 10390, 1994). The contents of organic C and total element concentrations were analysed in ground soil (Analysette 3 Spartan Pulverisette 0, Fritsch). Organic C was assessed using a dry combustion technique with a Leco RC-612 (ISO 10694, 1995). Total N was measured using the Kjeldahl method. Soils were digested in H_2SO_4 (1:24, w:v) with Kjeltabs ($K_2SO_4 + CuSO_4 \cdot 5H_2O$; Foss Tecator Digestor Auto) followed by distillation on Foss Tecator Kjeltec 2300 Analyser Unit (Foss Tecator Application Note AN 300). To determine the concentrations of total and available element forms, three soil extraction methods were applied. Total Ca, Cd, K, P, Pb and Zn were extracted by digestion of soil with hot concentrated $HClO_4$ (1:20, w:v; Foss Tecator Digestor Auto). Mobile forms of metals were extracted by shaking the soil samples for 1 h with 0.1 M $BaCl_2$ at pH 7.0 (1:5, w:v; Ca, Cd, K, Pb, Zn) and deionised water (1:5, w:v; Cd, Pb, Zn). The metal contents in the soil extracts were analysed with flame or graphite furnace atomic absorption spectrometry (Varian AA280FS; Varian AA280Z, GTA120). Total P was measured using the vanadate-molybdate method with a colorimeter (Hach-Lange DR 3800). Olsen P was extracted by shaking the soil with 0.5 M $NaHCO_3$ at pH 8.5 (1:10, w:v) and measured with an ion chromatograph (Dionex ICS-1100). Certified reference materials were used to estimate the quality of the metal analyses: CRM048-050 (RTC; total) and ISE-912 (WEPAL; $BaCl_2$-extractable).

2.2.2. Microbiological analyses

The soil basal respiration rate (BR) and microbial biomass (C_{MIC}), determined by substrate-induced respiration (SIR), were measured in soil samples adjusted to 50% WHC. Soil samples were incubated in gastight jars at 22 °C and the evolved CO_2 was absorbed in 0.2 M NaOH. The excess hydroxide was titrated with 0.1 M HCl following the addition of $BaCl_2$ and phenolphthalein as indicator. After the BR

measurements, a mixture of talcum and glucose monohydrate (4:1; 10 mg glucose g^{-1} dw soil) was added to the soil samples to measure substrate-induced respiration after 4 h of incubation as described above.

Urease activity (URE) was measured according to Kandeler (1996) using the unbuffered method. Soil samples were incubated at 37 °C for 2 h following the addition of urea solution. Released ammonium was extracted with KCl and determined by a modified Berthelot reaction, which is based on the reaction of sodium salicylate with ammonium in the presence of sodium dichloroisocyanurate and sodium nitroprusside at alkaline pH. Photometric measurements were performed at 690 nm with a Hach Lange DR 3800 spectrophotometer.

Arylsulfatase activity (ARY) was measured according to Strobl and Traunmüller (1996). Following the addition of acetate buffer (pH = 5.8) and p-nitrophenylsulfate, soil samples were incubated at 37 °C for 1 h. Nitrophenol released by arylsulfatase activity was extracted, colored with NaOH and determined at 420 nm using the Hach Lange DR 3800 spectrophotometer.

Plant roots were randomly collected from the heap soil samples and washed in tap water to remove remnants of soil. Phillips and Hayman (1970) method with minor modifications (Zubek et al., 2011) was used for staining of roots to visualize AMF mycelia. The abundance of AMF was assessed in fine living roots using a Nikon Eclipse 80i light microscope. Arbuscular mycorrhizal fungi colonization was analysed as reported by Trouvelot et al. (1986) and the following parameters were determined: mycorrhizal frequency (F_{AMF}), relative mycorrhizal root length (M_{AMF}) and relative arbuscular richness (A_{AMF}). F_{AMF} is expressed as a percentage ratio of the number of colonized roots to the number of total analysed sections of roots. M_{AMF} is a percentage ratio of the lengths of the colonized to those of the non-colonized parts of roots. A_{AMF} is as a percentage ratio of arbuscule abundance in the whole root system under study.

2.3. Calculations and statistical analysis

C_{MIC} was calculated according to the equation: C_{MIC} (μg g^{-1}) = 40.04x + 0.37, where x is the respiration rate given in μl CO_2 h^{-1} g^{-1} (Anderson and Domsch, 1978). The metabolic quotient (qCO_2) was calculated as respiration per biomass unit.

Statistical analysis was conducted in Canoco 5 (Ter Braak and Šmilauer, 2012) and R 3.3.3 (R Core Team, 2017). Prior to statistical analysis, continuous variables (both response and explanatory) were transformed where necessary with a logarithmic or exponential function to improve their normality or at least to remove skewness.

The main gradients in the plant species composition were detected separately for 65 heaps, the Trzebinia transect and the Bolesław transect using two ordination techniques: principal component analysis (PCA) or detrended correspondence analysis (DCA), depending on the gradient length (Ter Braak and Šmilauer, 2012). The analyses were based on the abundance data of the most frequent species, i.e., occurring in at least 20% of plots. The latent variables (ordination axes) obtained from PCA/DCA were used together with plant species richness as explanatory variables representing vegetation characteristics in subsequent multiple regressions (total percentage cover of vegetation was excluded due to its low variability).

In order to reveal the correlation structure in the soil physicochemical data, factor analysis with PCA method for factor extraction was performed separately for three datasets (65 heaps, Trzebinia transect, Bolesław transect). It is recommended that the number of variables in the factor analysis should be considerably lower than the number of cases; therefore, some variables were excluded from the input datasets. These variables were: silt content (it was almost the opposite of sand content) and Zn variables (they strongly correlated with Cd variables). In addition, $BaCl_2$-extractable Pb was excluded from the heap data, because its concentration in the soils of over two-thirds of the sampled sites was below the detection limit. The extracted factors were varimax-rotated for factor clarity and used in subsequent multiple regressions as explanatory variables representing the soil physicochemical properties.

Multiple regression was employed to explain the variability of soil microbial properties. As regards the mycorrhizal parameters, A_{AMF} was excluded from the analysis as it strongly correlated with M_{AMF} (Pearson's r was almost 1). In the case of the transect data, the first ordination axes representing the plant species composition strongly correlated with the first factors extracted in the factor analysis of soil physicochemical properties. The presence of these correlations resulted in an unacceptable level of multicollinearity, as shown by variation inflation factor calculated with the "car" package (Fox and Weisberg, 2011). Therefore, before multiple regression, the problematic plant variables (first ordination axes) were removed from the explanatory datasets; it was assumed that they were of secondary importance in relation to soil variables.

To find the best combination of explanatory variables for each dependent variable, the information-theoretic model selection procedure (Burnham and Anderson, 2004) was applied. The procedure employed the "dredge" function of the "MuMIn" package (Bartoń, 2016), which generates all possible candidate models and then ranks them according to Akaike information criterion (AIC); here, AICc (corrected AIC) was used due to the relatively small sample size.

3. Results

3.1. Heaps (large-scale study)

Heap soils were mostly sandy loam textured, alkaline (pH between 7.1 and 8.7) and with an average C:N ratio of about 15 (Table 1). They contained very large amounts of Ca and heavy metals. The total concentrations of the latter were over 400 (Cd), 20,000 (Pb) and 80,000 (Zn) mg kg^{-1} at the most polluted sites. Both total and available forms of heavy metals strongly varied between heaps, which was reflected in the high coefficients of variation (CV; standard deviations expressed as a percentage of the mean value), often far exceeding 100%. The variability of other soil physiochemical properties was much lower; CV was usually below 50%.

Factor analysis reduced soil physicochemical properties to a few independent latent variables (factors) that jointly explained 80% of the variance in the data (Table 2). Factor H1 represented the content of heavy metals (both total and available forms) in the soil with which the available P (Olsen P) was negatively related. Factor H2 reflected the content of organic matter and the availability of major nutrients (Ca, K). Factor H3 was an indicator of soil texture; it showed the content of the fine soil fraction (clay). Factors H4 and H5 represented the total soil P and soil alkalinity, respectively.

The number of plant species found on the heaps averaged 20 (per 4 m^2) and ranged from 4 to 33. Over half of the plots were fully covered (100%) with vegetation; the plant cover of the remaining plots ranged from 50 to 95%, with a predominance of high values. DCA performed on the most frequent plant species (Fig. 1) revealed a relatively high rate of compositional turnover (gradient length = 4.1 SD units), indicating that DCA was an appropriate method of ordination. The first two ordination axes explained 27% of the variance in the species data. According to Fig. 1, the sources of this variance were: the gradient of soil contamination with heavy metals ($DCA1_H$) and the gradient of soil pH ($DCA2_H$). The $DCA1_H$ gradient was responsible for the shift in the species composition

Table 1
Physicochemical properties of soils from heaps (large-scale study) and from transects (small-scale study).

	Heaps (N = 65)		Trzebinia transect (N = 25)		Bolesław transect (N = 25)	
Variable	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
pH	8.1 (0.3)	7.1–8.7	7.8 (0.2)	7.6–8.3	7.2 (0.4)	6.4–7.8
Sand (%)	66 (10)	43–82	61 (4)	54–68	72 (5)	62–79
Silt (%)	21 (8)	7–41	22 (4)	15–32	12 (5)	6–19
Clay (%)	14 (6)	6–42	16 (3)	12–22	16 (3)	10–21
Organic C (%)	5.2 (2.8)	1.6–13.8	4.4 (1.2)	1.8–6.4	3.1 (1.5)	1.3–5.8
Total Ca (g kg^{-1})	57 (34)	5–134	73 (33)	24–149	26 (31)	2–88
Total Cd (mg kg^{-1})	81 (98)	5–417	194 (134)	47–467	73 (75)	12–225
Total K (g kg^{-1})	1.59 (0.92)	0.46–4.08	2.14 (1.03)	0.79–4.04	0.82 (0.47)	0.25–1.74
Total N (%)	0.329 (0.133)	0.104–0.798	0.348 (0.097)	0.170–0.507	0.238 (0.101)	0.114–0.415
Total P (mg kg^{-1})	686 (138)	409–998	119 (87)	52–430	116 (58)	56–253
Total Pb (g kg^{-1})	2.6 (3.9)	0.1–21.7	6.8 (2.1)	4.1–13.5	0.6 (0.4)	0.2–1.2
Total Zn (g kg^{-1})	14.3 (17.4)	0.4–81.1	19.0 (13.8)	5.7–61.3	18.3 (22.0)	1.9–70.6
Olsen P (mg kg^{-1})	3.1 (1.8)	0.3–9.1	5.1 (1.1)	3.7–7.5	10.7 (3.7)	3.7–16.3
$BaCl_2$-extractable Ca (g kg^{-1})	2.29 (0.70)	0.79–4.51	1.37 (0.55)	0.54–2.61	0.82 (0.25)	0.42–1.33
$BaCl_2$-extractable Cd (mg kg^{-1})	4.5 (6.6)	0–38.3	5.8 (2.4)	2.4–10.3	5.1 (1.8)	2.1–8.4
$BaCl_2$-extractable K (mg kg^{-1})	101 (35)	33–167	54 (24)	16–117	66 (28)	27–126
$BaCl_2$-extractable Pb (mg kg^{-1})	0.5 (1.4)	0–10.4	2.1 (1.7)	0.6–8.7	0.5 (0.3)	0–1.1
$BaCl_2$-extractable Zn (mg kg^{-1})	43 (92)	0–562	20 (11)	5–47	120 (63)	37–272
H_2O-extractable Cd (mg kg^{-1})	0.036 (0.051)	0.004–0.362	0.075 (0.028)	0.038–0.137	0.039 (0.013)	0.014–0.066
H_2O-extractable Pb (mg kg^{-1})	0.75 (1.29)	0–7.31	1.29 (0.92)	0.41–4.14	0.40 (0.20)	0.06–0.98
H_2O-extractable Zn (mg kg^{-1})	4.08 (2.68)	0.48–9.48	3.80 (2.48)	0.87–9.17	3.16 (1.65)	1.08–6.36

SD – standard deviation.

Table 2
Factors extracted in factor analysis of soil physicochemical data obtained for heaps (H1–H5), the Trzebinia transect (T1-T4) and the Bolesław transect (B1–B4). For each factor, the amount of the variance explained (VE), a description and a list of variables having high loadings (>0.5) on the factor are given (variables with loadings >0.8 are in bold).

Dataset	Factor	VE (%)	Factor description	Variables with high loadings
Heaps (N = 65)	H1	27.3	Heavy metal content and availability	**Cd** (0.88), **Cd_{BC}** (0.87), **Cd_{W}** (0.86), **Pb** (0.85), Pb_W (0.74), P_{OLS} (−0.72)
	H2	21.2	Organic matter content and nutrient availability	**Ca_{BC}** (0.92), **N** (0.89), **C_{ORG}** (0.82), **K_{BC}** (0.81)
	H3	12.8	Texture (fine particle content)	**Clay** (0.96), Sand (−0.77), K (0.54)
	H4	9.6	P content	P (0.77)
	H5	9.1	Alkalinity	**Ca** (0.83), pH (0.74)
Trzebinia transect (N = 25)	T1	30.3	Cd content and nutrient content	**Cd_{W}** (−0.93), **Cd** (0.92), **P** (−0.88), Pb_W (−0.79), Ca (0.76), K (0.74), Cd_{BC} (0.56), P_{OLS} (−0.55)
	T2	22.8	Organic matter content and nutrient availability	**C_{ORG}** (0.97), **N** (0.96), **Ca_{BC}** (0.84), pH (−0.66), Clay (−0.55), K_{BC} (0.50)
	T3	15.3	Texture (fine particle content) and alkalinity	Sand (−0.77), Clay (0.74), pH (0.57), Ca (0.56), Pb (0.51)
	T4	12.0	Pb content and availability	**Pb_{BC}** (0.91), **Pb** (0.80)
Bolesław transect (N = 25)	B1	56.8	Heavy metal content and availability, organic matter content, nutrient availability and alkalinity	**Ca** (0.98), **Pb** (0.98), **Cd** (0.97), **K** (0.97), **C_{ORG}** (0.96), **N** (0.95), **pH** (0.85), **P** (0.81), Ca_{BC} (0.76), K_{BC} (−0.72), Cd_{BC} (0.70), P_{OLS} (−0.64), Sand (−0.62)
	B2	10.1	Heavy metal availability	**Pb_{W}** (0.87), Cd_W (0.79), P_{OLS} (0.50)
	B3	9.9	Texture (fine particle content)	**Clay** (0.90), Sand (−0.59)
	B4	9.0	Heavy metal availability	**Pb_{BC}** (0.95), Cd_{BC} (0.63)

BC – $BaCl_2$-extractable, W – H_2O-extractable, ORG – organic, OLS – Olsen.

from dry grasslands dominated by metal-tolerant plants (including *Festuca ovina*, *Silene vulgaris*, *Dianthus carthusianorum, Leontodon hispidus*, *Potentilla arenaria*, *Thymus pulegioides* and *Carex cariophyllea*) to metal-sensitive dry calcareous grasslands of the *Festuco-Brometea* class. The $DCA2_H$ gradient caused the latter communities to differentiate into pH-dependent variants.

Microbiological properties of heap soils (Table 3) were characterized by lower variability than physicochemical properties. This was indicated by lower CV values, ranging from 22 to 52%. The best regression models explained from 55 to 85% of the variance in the microbiological properties (Table 4). Soil pollution with heavy metals (factor H1; see Table 2) negatively affected soil microbial communities: with increasing concentrations of Cd and Pb (total and available), soil respiration, microbial biomass and enzymatic activity decreased, while the metabolic quotient increased (Table 4). In contrast, the content of organic matter and the availability of major nutrients in the soil (H2) had a positive effect on soil microbes. This effect was much stronger than that of factor H1 as shown by higher β coefficients. There were few significant relationships between microbial community parameters and other factors describing the soil physicochemical environment. Among the strongest, there was a negative relationship between the content of clay particles (H3) and arylsulfatase activity and a positive one between pH (H5) and qCO_2. As for the impact of vegetation on soil microbes, it was observed in only one case: arylsulfatase activity increased with the increasing number of plant species (SR_H) and with the compositional change represented by the $DCA1_H$ axis. The level of mycorrhization was influenced by independent variables in a similar way to microbial communities, but the response to physicochemical properties was weaker than the response to vegetation parameters.

3.2. Heap-fallow transects (small-scale study)

The two heap-fallow transects differed in terms of many soil physicochemical properties. In general, the soils from the Trzebinia transect were less sandy, more alkaline and richer in basic nutrients, such as C, N, K and Ca, than soils from the Bolesław transect

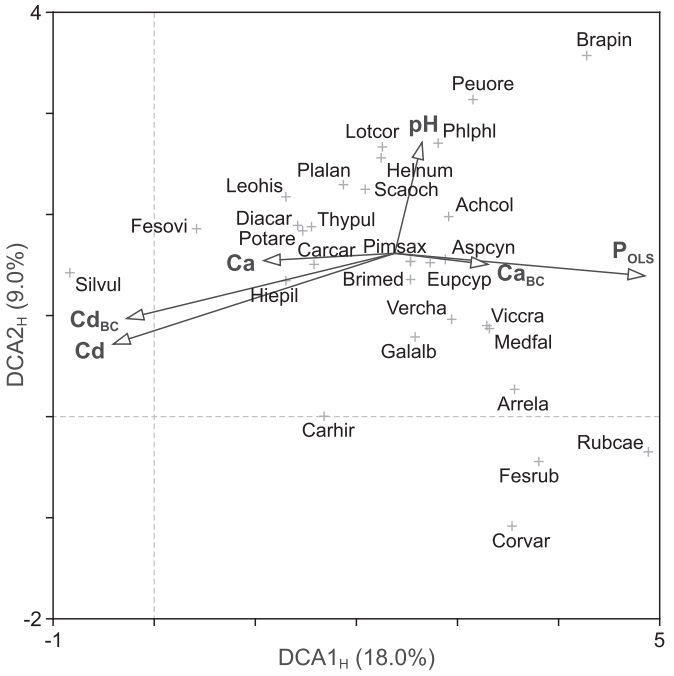

Fig. 1. Ordination diagram based on DCA of plant species data collected from 65 heaps. The analysis included the most frequent species (34) but, for clarity, only important differential ones (weight higher than 10%) were displayed. For the purpose of interpretation, forward-selected environmental variables were passively projected into the ordination space. Explanation of abbreviations used for species names: Achcol, *Achillea collina*; Arrela, *Arrhenatherum elatius*; Aspcyn, *Asperula cynanchica*; Brapin, *Brachypodium pinnatum*; Brimed, *Briza media*; Carcar, *Carex caryophyllea*; Carhir, *Carex hirta*; Corvar, *Coronilla varia*, Daucar, *Daucus carota*; Diacar, *Dianthus carthusianorum*; Eupcyp, *Euphorbia cyparissias*; Fesovi, *Festuca ovina*; Fesrub, *Festuca rubra*; Galalb, *Galium album*; Helnum, *Helianthemum nummularium*; Hiepil, *Hieracium pilosella*; Leohis, *Leontodon hispidus*; Lotcor, *Lotus corniculatus*; Medfal, *Medicago falcata*; Peuore, *Peucedanum oreoselinum*; Phlphl, *Phleum phleoides*; Pimsax, *Pimpinella saxifrage*; Plalan, *Plantago lanceolate*; Potare, *Potentilla arenaria*; Rubcae, *Rubus caesius*; Scaoch, *Scabiosa ochroleuca*; Silvul, *Silene vulgaris*; Thypul, *Thymus pulegioides*; Vercha, *Veronica chamaedrys*; Viccra, *Vicia cracca*.

(Table 1, Supplement 1). They also contained much more Cd and Pb (both total and available), but not Zn; in the Bolesław transect, soil contamination with Zn was similar to that in the Trzebinia transect, or even higher, considering the $BaCl_2$-extractable form (Table 1, Supplement 1). The transects did not differ in the total P content, but this element was more available in the Bolesław transect. The data from the transects were less variable than the heap data; only for the total Ca, Cd and Zn measured in the Bolesław transect, CV exceeded 100%.

Factor analysis on the Trzebinia soil physicochemical properties resulted in four factors accounting for 80% of the variance in the data (Table 2). Factor T1 represented the total Cd, Ca and K, with which the contents of P and H_2O-extractable heavy metals negatively correlated. Factor T2 represented the content of organic matter and $BaCl_2$-extractable Ca. Factor T3 mainly reflected the fine soil fraction (clay), and factor T4 – Pb pollution (total and $BaCl_2$-extractable forms). Also, the Bolesław data were reduced by factor analysis to four factors (Table 2); they jointly explained 86% of the variance. Factor B1 combined the total content of heavy metals, organic matter and all major nutrients. Factors B2 and B4 represented H_2O- and $BaCl_2$-extractable heavy metals, and factor B3, as in the case of Trzebinia transect, the clay content.

The values of variables represented by the above-described first factors, T1 and B1, were strongly related to the position in the heap-fallow gradient. In the case of the Trzebinia transect, the total content of Cd, Ca and K reached the highest values at the heap plots and gradually decreased with the distance from the heaps (Fig. 2). Clearly, the opposite trend was exhibited by the variables negatively correlated with the factor (H_2O-extractable heavy metals and total P). In the case of the Bolesław transect, the heap plots were characterized by high and the fallow plots by low contents of total heavy metals and major nutrients, and there was no gradual change between the heap and fallow (Fig. 2, Supplement 1). The values of variables contributing to other factors were not related to the heap-fallow gradient both within the Trzebinia and Bolesław transect.

The transects differed in vegetation parameters. The number of vascular plant species (per 1 m^2) in the Trzebinia transect averaged 16 and ranged from 10 to 24, while in the Bolesław transect it averaged 10 and ranged from 6 to 15. Total percentage cover of vegetation was lower than 100% in only 5 out of 25 plots of the Trzebinia transect (minimum value = 80%); in the Bolesław transect, there were 16 such plots (minimum value = 30%). According to DCA, compositional gradients in the species data from the transects were shorter than that determined for the species data from the heaps. They were 2.4 SD (Trzebinia transect) and 3.4 SD (Bolesław transect), that is, they fell into the range (1.5–4 SD) for which both ordination techniques, unimodal (DCA) and linear (PCA), work reasonably well. Here, PCA was chosen because it produced sample scores with better statistical distributions. The first and second ordination axis explained jointly the same amount of variance (ca. 50%) in the Trzebinia and Bolesław species data (Fig. 3). For both transects, the transition in the species composition along the first axes ($PCA1_T$, $PCA1_B$) was strongly related to the heap-fallow heavy-metal contamination gradient (see also Fig. 2) and was of a similar nature. Namely, the heap area was overgrown by loose dry grassland with metal-tolerant plants (generally the same as those mentioned when explaining the $DCA1_H$ gradient; see above), while the fallow area was dominated by denser mixed vegetation with grassland, meadow and ruderal species, such as *Achillea collina*, *Lotus corniculatus*, *Galium album*, *Brachypodium pinnatum*, *Carex hirta*, *Leontodon hispidus*, *Agrostis stolonifera* and *Picris hieracioides*.

Table 3
Soil microbiological properties and AMF (arbuscular mycorrhizal fungi) root colonization parameters measured for heaps (large-scale study) and for transects (small-scale study).

	Heaps (N = 65)		Trzebinia transect (N = 25)		Bolesław transect (N = 25)	
Variable	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
BR – basal soil respiration (μM CO_2 g^{-1}_{dw} 24 h^{-1})	2.39 (0.64)	1.13–4.04	3.09 (0.66)	2.13–4.46	1.88 (1.20)	0.52–3.84
C_{MIC} – microbial biomass (mg g^{-1}_{dw})	0.579 (0.208)	0.148–0.940	0.622 (0.165)	0.298–0.938	0.457 (0.213)	0.172–0.922
*q*CO2 – metabolic quotient (μg C–CO_2 mg C^{-1}_{MIC} h^{-1})	2.24 (0.71)	1.30–4.80	2.53 (0.48)	1.77–3.58	1.92 (0.70)	0.93–3.95
URE – Urease activity (μg N g^{-1}_{dw} h^{-1})	48.9 (17.7)	6.6–88.0	41.9 (17.8)	7.7–86.6	23.9 (12.9)	0.3–52.5
ARY – Arylsulfatase activity (μg pNP g^{-1}_{dw} h^{-1})	261 (130)	10–476	243 (95)	48–462	80 (33)	34–141
F_{AMF} – mycorrhizal frequency (%)	84 (19)	33–100	nd	nd	nd	nd
M_{AMF} – relative mycorrhizal root length (%)	46 (21)	6–82	nd	nd	nd	nd
A_{AMF} – relative arbuscular richness (%)	41 (22)	4–82	nd	nd	nd	nd

SD – standard deviation; nd – not determined.

Table 4
Multiple regression models that best explain the variability in soil microbiological properties measured for 65 heaps (all models were significant at $P < 0.001$). Standardized regression coefficients (β) were calculated only for those dependent variables that were included in a given model.

	BR	C_{MIC}	qCO_2	URE	ARY	F_{AMF}	M_{AMF}
$R^2_{adjusted}$	0.70	0.85	0.55	0.78	0.65	0.62	0.59
F	49.0	115.9	19.6	111.0	18.0	21.4	16.3
β coefficients							
H1	−0.168*	−0.459***	0.586***	−0.402***	−0.219*	−0.356**	−0.261*
H2	0.810***	0.796***	−0.358***	0.798***	0.501***	0.151(*)	0.222*
H3	0.159*	–	–	–	−0.216**	–	
H4	–	–	0.164(*)	–	0.185*	–	−0.129
H5	–	−0.132*	0.274**	–	−0.191*	−0.252**	−0.167(*)
SR_H	–	–	–	–	0.290**	0.306**	0.238*
$DCA1_H$	–	–	–	–	0.238*	0.357**	0.420**

Explanation of dependent variables: H1–H5 – factors extracted in factor analysis of soil physicochemical data (see Table 2); SR_H – plant species richness; $DCA1_H$ – plant species composition represented by $DCA1_H$ axis (see Fig. 1). Significance level: *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$.

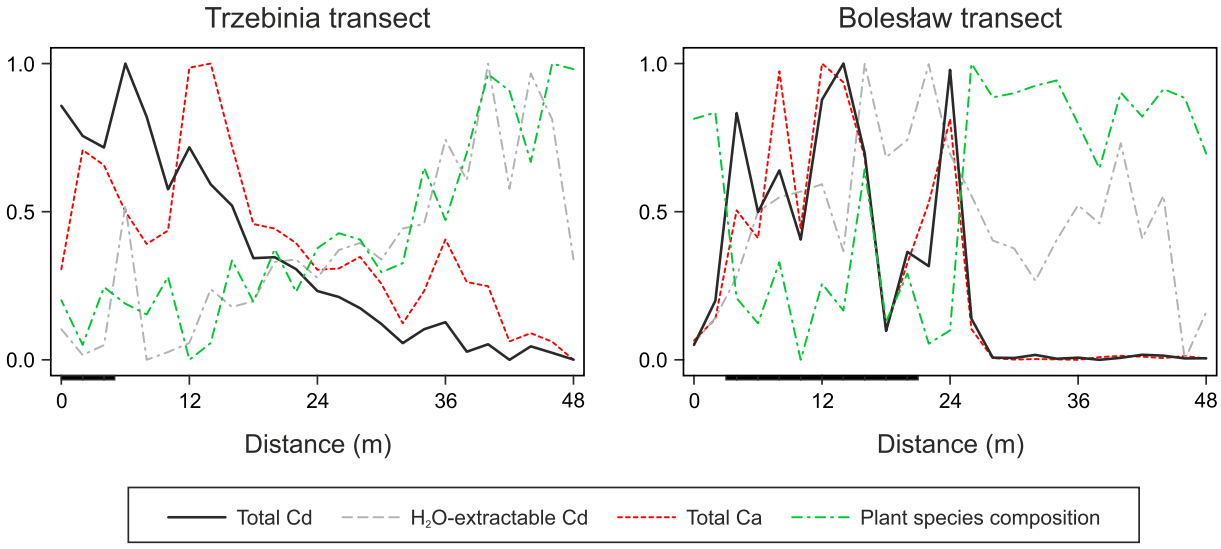

Fig. 2. Variability of selected soil physicochemical properties and plant species composition (represented by PCA1; see Fig. 2) in the Trzebinia and Bolesław transect. The variables were scaled between 0 and 1. The black bar at the bottom of the graphs indicates the part of the transect that runs through the heap area.

The fallow vegetation differed between the transects more than the heap vegetation. In addition, its variability along the second axis apparently had different causes: organic matter gradient in the case of $PCA2_T$ and heavy metal availability gradient in the case of $PCA2_B$ (Fig. 3). For both transects, there was no clear difference between the heap and fallow plots in terms of the number of plant species.

Soil microbial properties, especially enzymatic activity, reached on average higher values in the Trzebinia transect than in the Bolesław transect (Table 3, Supplement 1). The amount of variance in these properties explained by multiple regressions (best models) was relatively high (Table 5); it ranged from 64 to 91% (Trzebinia transect) and from 58 to 94% (Bolesław transect). In both transects, the content of organic matter combined with the content (total or available) of major nutrients had the strongest impact on microbial communities (Table 5): factors T2 and B1 positively affected almost all microbial parameters (the only negative relationship was between qCO_2 and T2). The adverse effect of heavy metals on soil microbes was most evident in the Trzebinia transect in the negative response of microbial biomass and arylsulfatase activity to factor T1 (total Cd), and the positive response of qCO_2 to factors T1 and T4 (available and total Pb). In the Bolesław transect, the negative relationship between arylsulfatase activity and factor B4 ($BaCl_2$-extractable Pb and Cd) was the only example of such an effect. In both transects, the third factor (T3, B3), representing the content of clay particles, negatively influenced soil microbial communities as shown by the signs of the detected relationships (Table 5). In contrast to soil physicochemical properties, the vegetation parameters (those included in the analysis) had a marginal effect on soil microbes; the only statistically significant ($P < 0.05$) relationship was the positive one between urease activity and plant species richness (SR_T).

4. Discussion

Total concentrations of heavy metals in the soil of heaps left after historical Zn–Pb mining were very high, usually considerably exceeding allowable limits set by Polish law for forested areas and wastelands (10 mg Cd kg^{-1}, 500 mg Pb kg^{-1}, 1000 mg Zn kg^{-1}) as well as arable lands, pastures and meadows (2–5 mg Cd kg^{-1}, 100–500 mg Pb kg^{-1}, 300–1000 mg Zn kg^{-1}; Rozporządzenie Ministra Środowiska, 2016). The transect study showed that these concentrations, particularly of Cd and Zn, were much lower outside the heaps (i.e., in the fallow area), but even there they remained elevated in relation to the standards (see also Stefanowicz et al., 2014). High background values of heavy metals in the study region, resulting from geology and centuries-old mining and smelting activities (Godzik and Woch, 2015), certainly contributed to the result. However, the main reasons for the high level of pollution of

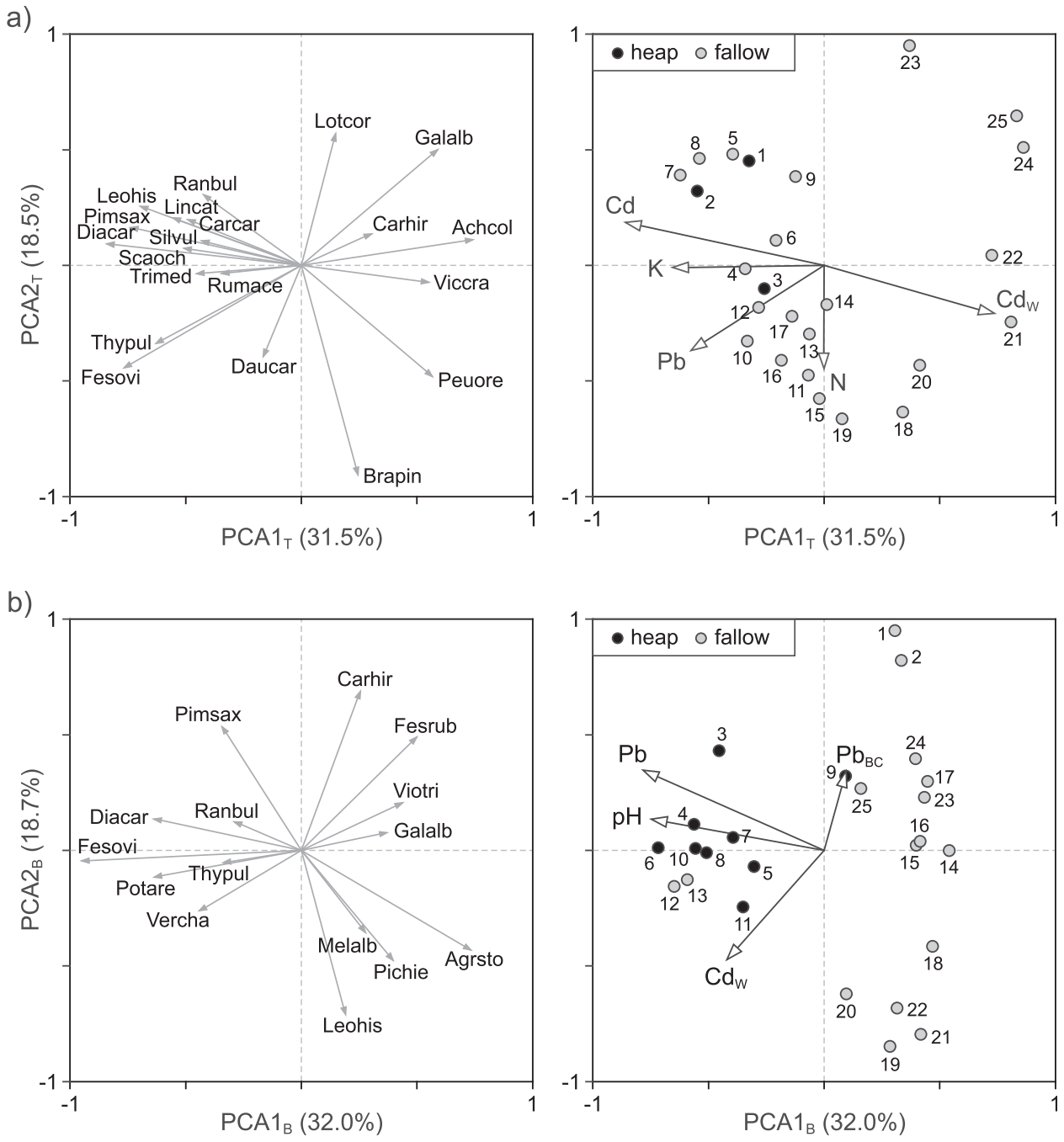

Fig. 3. Ordination diagrams of species (left) and samples (right) based on PCA of plant species data collected from a) the Trzebinia transect and b) the Bolesław transect. The analysis included the most frequent species (23 and 18, respectively) but, for clarity, only important differential ones (weight higher than 10%) were displayed. For the purpose of interpretation, forward-selected environmental variables were passively projected into the ordination space (right diagrams). Explanation of abbreviations used for species names (only those that were not given in Fig. 1): Agrsto, *Agrostis stolonifera*; Lincat, *Linum catharticum*; Melalb, *Melandrium album*; Ranbul, *Ranunculus bulbosus*; Rumace, *Rumex acetosella*; Trimed, *Trifolium medium*; Viotri, *Viola tricolor*.

the fallow soil were most probably the migration of heavy metals down the heap slopes with surface runoff and the accidental spread of the mining waste material during past agricultural works (e.g., ploughing). The Trzebinia transect is where the latter factor played an important role. The heap was gradually destroyed (levelled) over time by agricultural machinery, and although its large part ceased to be conspicuous, it still affects soil properties and organisms. This explains why several plots marked as fallow have a similar plant species composition as the heap plots (Fig. 3a). In conclusion, the objects under investigation are hotspots of metal pollution that tends to disperse. Given that heavy metals easily enter the food chains via plants growing on and around the heaps (Peralta-Videa et al., 2009; Stefanowicz et al., 2016), the range of this dispersion and the associated environmental impact can be quite substantial.

Soil pollution in this study was not only high but also highly variable (there were three orders of magnitude differences in the total soil concentrations of heavy metals). Therefore, we expected that its negative impact on soil microbial communities would be observed. It happened, but only where the gradient of soil pollution was fairly independent of the gradients of other soil properties and

Table 5
Multiple regression models that best explain the variability in soil microbiological properties measured for the Trzebinia and Bolesław transects (all models were significant at $P < 0.001$). Standardized regression coefficients (β) were calculated only for those dependent variables that were included in a given model.

	BR	C_{MIC}	qCO_2	URE	ARY
Trzebinia transect					
$R^2_{adjusted}$	0.79	0.89	0.70	0.64	0.91
F	23.8	65.0	15.2	11.1	56.1
B coefficients					
T1	0.253*	−0.148*	0.562***	–	−0.333***
T2	0.820***	0.881***	−0.433***	0.578***	0.869***
T3	−0.179(*)	−0.297***	0.195(*)	−0.368*	−0.120(*)
T4	0.241*	–	0.460***	–	−0.119(*)
SR_T	–	–	–	0.313*	–
$PCA2_T$	–	–	–	−0.271(*)	–
Bolesław transect					
$R^2_{adjusted}$	0.94	0.83	0.79	0.58	0.78
F	178.8	61.1	43.8	17.3	28.2
β coefficients					
B1	0.962***	0.903***	0.796***	0.686***	0.836***
B3	0.119*	−0.180*	0.415***	−0.375*	−0.323**
B4	–	–	–	–	−0.246*

Explanation of dependent variables: T1-T4 and B1−B4 − factors extracted in factor analysis of soil physicochemical data (see Table 2); SR_T − plant species richness; $PCA2_T$ − plant species composition represented by $PCA2_T$ axis (see Fig. 2). Significance level: *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$.

vegetation parameters, i.e., in the absence of confounding factors. Factor H1, extracted from the heap data, was composed almost exclusively of heavy metal variables and as such it reflected the level of environmental stress and nothing else. Not surprisingly, it negatively affected all microbiological parameters except for qCO_2 (but note that the positive sign of the β coefficient in the qCO_2 regression model is a reflection of the negative response of soil microbes; see below). Similarly, factor T4 − an indicator of Pb contamination in the Trzebinia transect − exerted a detectable, although much weaker, negative effect on soil microbes (manifested by the increased qCO_2). In contrast to H1 and T4, the first factor for the Bolesław transect (B1) represented the joint variability of the soil content of heavy metals and essential macronutrients (Ca, K, C_{ORG}, N, P) as well as soil pH. In this situation, the effects of metal toxicity were not detected, because they were either largely reduced (due to immobilization of heavy metals by organic matter and gangue minerals) or compensated (due to better nutrition conditions). This phenomenon has been commonly reported in the field-study literature (Azarbad et al., 2013; Chodak et al., 2013; Ciarkowska et al., 2014; Guo et al., 2017; Stefanowicz et al., 2012). The first factor for the Trzebinia transect (T1) was an intermediate case. Although it combined, like B1, heavy metal and macronutrient variables, it maintained the ability to adversely affect soil microbes (we found a negative effect on microbial biomass and arylsulfatase activity, and a positive one on qCO_2). This result can be explained by the higher level of soil pollution in the Trzebinia transect and the lack of the toxicity-mitigating effect of organic matter (C_{ORG} and N were represented by another factor).

As for the concentrations of available heavy metals, their impact on soil microbial communities was difficult to determine. This was due to the fact that they often co-varied with the total heavy metal concentrations. In the heap soils, total and available forms of Cd and Pb were strongly positively correlated with each other (H1). A similar relationship existed between the total and $BaCl_2$-extractable Pb in the Trzebinia transect (T4). In this transect, the opposite relationships were also observed: the total Cd negatively correlated with H_2O-extractable Cd and Pb (T1), which means that the concentrations of the latter were higher in the fallow than within the heap (Fig. 2). This outcome confirms the assumption that heavy metals can migrate gravitationally with rainwater down the heap slopes to the surrounding area. The lower concentration of total Ca in fallow soil resulting from a lower content of gangue minerals, dolomite and calcite, may also contribute to this effect (Cabala et al., 2009; Stefanowicz et al., 2014). The only case where the variability of available forms of heavy metals was independent of the variability of their total forms was the Bolesław transect. Here the impact of the former on soil microbes could be estimated. What's more, this estimation could be done separately for the $BaCl_2$-and H_2O-extractable forms, because they were in different factors. Multiple regression showed that microbial communities hardly responded to the increased availability of heavy metals in the Bolesław transect (we found only a weak negative effect on arylsulfatase activity). The result is surprising, since soil heavy metal toxicity is considered to be a function of the concentration of heavy metal available forms (Legrand et al., 2005; Giller et al., 2009). On the other hand, there is some evidence that metal forms measured in soils as "available" do not necessarily reflect the actual availability of these elements for plants and soil organisms (Legrand et al., 2005; Kabata-Pendias and Mukherjee, 2007).

Soil respiration itself is an unreliable indicator of the microbial community health. High respiration rate may indicate increased microbial growth resulting from favourable habitat conditions or increased energy expenditure for maintenance, including detoxification, under environmental stress (Zhang et al., 2010). Metabolic quotient, qCO_2, which expresses the amount of CO_2 released per unit of biomass, helps to understand which processes (productive or non-productive) prevail in microbial communities. According to Nielsen and Winding (2002), qCO_2 is high when the stress level is high. Such a positive relationship has been repeatedly confirmed in the field studies conducted in heavy metal contaminated (Stefanowicz et al., 2010; Yao et al., 2003; Zhang et al., 2010) and in other post-industrial areas: recently, Woch et al. (2018) showed increased qCO_2 at high concentrations of C, S, P, Mn, alkali and alkaline earth metals, which characterized coal mining or combustion waste (coal sludge and ash). Our study, demonstrating that qCO_2 more strongly (high β coefficients) and less ambiguously (β always with a positive sign) responded to heavy metal stress than soil respiration and microbial biomass alone, is in line with the above literature.

Although heavy metal pollution is a meaningful agent shaping soil microbial communities, other soil physicochemical properties, such as organic matter, pH and texture, are often even more important. They can counteract the negative effects of heavy metals, thus hindering the assessment of ecological risk (Azarbad et al., 2013; Chodak et al., 2013; Ciarkowska et al., 2014; Guo et al., 2017; Stefanowicz et al., 2012). In this study, the impact of organic matter on soil microbes (positive) was dominant and far outweighed that of heavy metals. This is evident from the fact that all three factors representing C_{ORG} and N (H2, T2 and B1) were significant predictors of all microbiological parameters and had the highest explanatory power (highest β coefficients) despite the relatively small variability of the soil content of these elements. In the case of the Bolesław transect, organic matter was most likely what suppressed the detrimental effect of heavy metal pollution, with which it positively correlated. The positive relationship between the soil content of organic matter (or simply its main components, C and N) and soil microbial indicators is a well-known phenomenon. First, organic matter provides heterotrophic microbial communities with a variety of substrates for their growth (Azarbad et al., 2013; Chodak et al., 2013; Moreno et al., 2009). Second, it significantly influences the behaviour of elements, as it is a rich source of negative charges (Kabata-Pendias, 2011; Tack, 2010). In heavy metal-polluted soils, it tends to adsorb heavy metals, which reduces their availability and thus toxicity to

organisms (Azarbad et al., 2013; Lair et al., 2007; Moreno et al., 2009). Organic matter is 6–13 times more effective sorbent for heavy metals than soil minerals (Lair et al., 2007). The retention mechanisms of elements by organic matter include weak physical forces, ion exchange, the formation of inner-sphere complexes and precipitation (Lair et al., 2007; Tack, 2010). Soils rich in organic matter can bind metals efficiently not only in alkaline, but also in acidic conditions; however, bonding capacity depends on metal (Kabata-Pendias, 2011; Tack, 2010). Pb is fixed very strongly, while Cd and Zn are fixed rather fairly and slightly, respectively (Kabata-Pendias, 2011). Many authors stressed the importance of soil pH and texture for soil microbial communities (Lauber et al., 2008; Chodak and Niklińska, 2010; Azarbad et al., 2013; Chodak et al., 2013; Guo et al., 2017). These properties may strongly influence soil microbes either directly or indirectly. For example, they can control the mobility and thus toxicity of heavy metals, as clay minerals enhance, like organic matter, soil sorption capacity, while pH affects the availability of sorption sites (Kabata-Pendias and Mukherjee, 2007). Chodak et al. (2013) observed that the structure and diversity of microbial communities in forest soils contaminated with heavy metals depended mainly on soil pH. Lauber et al. (2008) found that soil pH and texture were the best predictors of bacterial community composition across different land use types. Chodak and Niklińska (2010) reported that post-mining loamy sands not only supported more active microbial communities than sands but also fostered the accumulation of plant-derived organic matter due to the building of organo-mineral complexes resistant to microbial degradation. In our study, the effects of clay content (factors H3, T3 and B3) and pH (factor H5) were relatively weak and, interestingly, consistently negative, i.e., the reverse of those reported in the literature. Such a result might be due to the low variability of these properties (most soils were slightly alkaline and sandy loam textured) and the influence of clay and pH covariates that were not included in this study, e.g., the concentration of other toxic elements (As, Tl; Cabala et al., 2009).

Vegetation has a huge impact on the soil environment through root growth, selective uptake of nutrients, secretion of various substances and supply of dead organic matter in the form of leaf litter and root residues (Bardgett et al., 2014; Haichar et al., 2014; Metcalfe et al., 2011). Therefore, it is one of the most important drivers of soil microbial communities. This is clearly visible at the first stages of plant succession in post-industrial areas with degraded and often heavily contaminated soil; the colonization of bare soil patches by vegetation results in a marked increase in microbial activity and biomass regardless of the type of soil or pollution (Stefanowicz et al., 2015; Borymski et al., 2018). Not only the presence or biomass of plants is essential for soil microbes but also their species composition, as plant species represent different functional groups and vary considerably in functional traits (Johnson et al., 2003; Orwin et al., 2010; Scharfy et al., 2011). For example, graminoids have higher tissue density, total and below-ground biomass, N-use efficiency, longer leaf lifespan and lower leaf thickness, nutrient concentration and litter decomposability than forbs, which has implications for the soil environment (Johnson et al., 2003; Orwin et al., 2010; Scharfy et al., 2011). Moreover, plant species from the same functional group can also differ significantly in their physiological traits. Niemann et al. (1992) reported that grasses with low relative growth rate contained more cell wall materials, such as lignin, hemicellulose, cellulose, and polysaccharide-bound ferulic acid, when compared to fast-growing species. In our study, the plant species composition was highly variable. Significantly, the analysis of all three datasets showed that the largest compositional shift was related to the heavy metal gradient and followed the same pattern. Namely, the most polluted heaps and the most polluted parts of the transects were overgrown by loose heavy metal grassland dominated by a grass *Festuca ovina*; at the lower level of pollution, the more dense, metal-sensitive vegetation developed, which was representative of a given location: dry calcareous grassland composed mainly of a grass *Brachypodium pinnatum* or meadow-ruderal communities, sometimes with an admixture of woody plants (Woch et al., 2016; Woch, 2017). Since the variability in the plant species composition was strongly correlated with the soil pollution gradient (for this reason, $PCA1_T$ and $PCA1_B$ were excluded from the multiple regression analysis) we could not determine its 'pure' effect on soil microbes. We can only speculate that it strengthens rather than weakens the negative impact of heavy metals. *F. ovina*, which dominated in highly polluted sites, is a slow-growing species producing litter rich in hard-to-decompose lignin and cellulose (Koukoura et al., 2003; Niemann et al., 1992). In contrast, *B. pinnatum*, which occurred commonly in less polluted sites (Woch et al., 2016; Woch, 2017), is an expansive fast-growing plant with a higher quality of litter, in terms of N and lignin content, than *F. ovina* (Niemann et al., 1992; Orwin et al., 2010). In addition, *B. pinnatum* was often accompanied or replaced by other highly productive species, including invasive *Solidago canadensis*, and legumes, for example, *Lotus corniculatus*, *Anthyllis vulneralia* or *Vicia cracca* (Woch et al., 2016). Such assemblages provide much better support for soil microbes than those based on *F. ovina*.

Each plant species is unique in terms of the quality of the litter it produces, and the type of chemical compounds it releases into the soil environment (Paterson et al., 2008; Bugg et al., 2011; Haichar et al., 2014; Mincheva et al., 2014). Hence, the more species in a plant community, the higher the availability of different carbon substrates and other nutrients that can be used by soil microbes. This should be reflected in the positive response of soil microbial communities to the increasing plant species richness. Such a response was found in this work, but it was weaker than expected (it concerned only enzymatic activity and AMF colonization). This may be explained by the fact that the most pronounced effects of species richness are often observed in the range from one to a few species per species mixture (Loranger-Merciris et al., 2006; Milcu et al., 2010). In our study, there were only multispecies assemblages, which usually consisted of much more than five species per plot.

It should be kept in mind that plants and soil microbes may also affect each other indirectly – by changing the availability of soil contaminants. Plant species with the ability to hyperaccumulate heavy metals may reduce the concentrations of their soluble forms and thus support some functions of microbial communities (Gremion et al., 2004). Both plant roots and bacteria may release siderophores – low molecular mass compounds that are able to form stable complexes with Fe and other metals of environmental concern, for example Cd, Pb and Zn (Rajkumar et al., 2010; Reichman and Parker, 2005). Binding of the siderophore to a metal increases its solubility (Rajkumar et al., 2010). The modification of metal availability in the rhizosphere may also be related to the release of protons, organic acids and peptides via roots (Greger, 2005). Moreover, Moshiri et al. (2019) revealed that AMF affect biogeochemical fractions of metals and their accumulation in plant tissues. Plants colonized by AMF stabilize some metals in roots and translocate less amounts to shoots (Motaharpoor et al., 2019; Rohani et al., 2019). AMF also increase the activity of some antioxidant enzymes and concentrations of phenolic compounds, which result in the reduction of plant oxidative stress in heavy metal contaminated soils (Rohani et al., 2019). The occurrence of the above-described phenomena may be another reason for the lack of a clear response of soil microbes to the variability of vegetation parameters.

5. Conclusions

In this study, the microbiological parameters were measured in a wide range of total concentrations of Cd, Pb and Zn in the soil, from moderate (near the environmentally acceptable limits) to extremely high (i.e., three orders of magnitude higher). Despite the steep gradients of soil pollution, the toxic effects of heavy metals were weaker than the effects of some other soil properties. Soil microbes much more strongly responded to the variability of the organic matter content (positively), although this variability was quite low. Where the soil organic matter co-varied with the pollution variables, the effect of the latter was completely eliminated or compensated. An additional difficulty in assessing the impact of heavy metals on soil microbes was that they substantially altered the plant species composition, which is not indifferent to underground organisms. In highly polluted sites, loose metal-tolerant grasslands developed, which were dominated by slow-growing species, mostly *Festuca ovina*. Less polluted sites were overgrown by denser vegetation composed by more productive species, including *Brachypodium pinnatum*. Due to the strong correlation between the plant species composition and heavy metal pollution, it could not be unambiguously determined whether microbial communities responded more to soil toxicity or the resulting quality of plant support. In conclusion, environmental risk assessment of metal polluted sites using microbiological indicators should be carried out with caution, as the impact of heavy metals is strongly dependent on the context, i.e., on the basic soil properties with which these metals are correlated and on the effect they have on plant community structure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.124922.

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